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(54) Title: AGP–1 FUSION PROTEIN COMPOSITIONS AND METHODS

(57) Abstract

The present invention relates to Fc–AGP–1 fusion protein compositions, methods of preparation of such compositions and uses thereof. In particular, the present invention relates to a genetic or chemical fusion protein comprising the Fc immunoglobulin region, derivative or analog fused to the N–terminal portion of the AGP–1 protein, derivative or analog.

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AGP-1 FUSION PROTEIN COMPOSITIONS AND METHODS

Field of the Invention

The present invention relates to AGP-1 fusion protein compositions and methods of preparation and use thereof.

Background of the Invention

The availability of recombinant proteins for therapeutic use has led to advances in protein modifications in order to enhance or improve the properties of such proteins as pharmaceutical agents. Such modifications can provide enhanced protein protection and decreased degradation by reducing or eliminating proteolysis. Additional advantages include, under certain circumstances, increasing the stability, circulation time, and the biological activity of the therapeutic protein. A review article describing protein modifications is Francis, *Focus on Growth Factors* 3:4-10 (May 1992) (published by Mediscript, London, UK).

One such modification is the use of an Fc region of an immunoglobulin molecule. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain, known as "Fc" which provides the link to effector functions such as complement or phagocytic cells. The Fc portion of an immunoglobulin has a long plasma half-life, whereas the Fab is short-lived. (Capon, *et al.*, Nature 337, 525-531 (1989)).

Therapeutic protein products have been constructed using the Fc domain to provide longer half-life or to incorporate functions such as Fc receptor binding, protein A binding, complement fixation and placental transfer which all reside in the Fc proteins

of immunogobulins. *Id.* For example, the Fc region of an IgG1 antibody has been fused to the N-terminal end of CD30 ligand (CD30-L), a molecule which binds CD30 receptors expressed on Hodgkin's Disease tumor cells, anaplastic lymphoma cells, T-cell leukemia cells and other malignant cell types. See, U.S. Patent No. 5,480,981. IL-10, an anti-inflammatory and antirejection agent has been fused to murine Fcγ2a in order to increase the cytokines short circulating half-life. (Zheng *et al.*, The Journal of Immunology, 154, 5590-5600 (1995)). Studies have also evaluated the use of tumor necrosis factor receptor linked with the Fc protein of human IgG1 to treat patients with septic shock. (Fisher *et al.*, N. Engl. J. Med., 334: 1697-1702 (1996); Van Zee *et al.*, The Journal of Immunology, 156: 2221-2230 (1996)). Fc has also been fused with CD4 receptor to produce a therapeutic protein for treatment of AIDS. See, Capon *et al.*, Nature, 337:525-531 (1989). In addition, the N-terminus of interleukin-2(IL-2) has also been fused to the Fc portion of IgG1 or IgG3 to overcome the short half life of IL-2 and its systemic toxicity. See, Harvill *et al.*, Immunotechnology, 1, 95-105 (1995).

A Type II transmembrane protein of the tumor necrosis factor (TNF) family has been identified and observed to induce apoptosis in certain tumor cell lines, but not in normal cell lines. This protein has been referred to as TRAIL (Wiley et al. Immunity 3, 673-682 (1995)), Apo-2 (Pitti et al. J. Biol. Chem. 22, 12687-12690 (1996) and AGP-1 (WO97/46686) and is referred to herein as AGP-1. AGP-1 has been studied as a soluble protein which lacks transmembrane and intracellular domains and is most advantageously used as a therapeutic in this form. However, the apoptotic

activity of soluble AGP-1 is too low for it to be useful as a therapeutic.

Consequently, there exists a need to develop AGP-1 protein compositions for clinical application. Such development would include AGP-1 protein compositions which achieve increased biological activity, decreased degradation, increased stability and increased circulation time. The present invention provides such compositions.

Summary of the Invention

The present invention relates to Fc-AGP-1 fusion protein compositions, methods of preparation of such compositions and uses thereof. In particular, the present invention relates to a fusion protein comprising an Fc protein, or variant, fragment or derivative thereof, fused to the N-terminal portion of an AGP-1 protein, or variant, fragment, or derivative thereof. Unexpectedly, as described herein, a fusion of an Fc protein to the N-terminus of a soluble AGP-1 protein demonstrates enhanced biological activity compared to an unmodified soluble AGP-1 protein. Such unexpected advantages from the Fc modification to AGP-1 protein would be advantageous in that these changes contribute to lower doses required or less frequent dosing. Thus, as described below in more detail, the present invention has a number of aspects relating to the modification of proteins via fusion of an Fc region to an AGP-1 protein (or variant, fragments or derivative thereof), as well as, specific modifications, preparations and methods of use thereof.

The present invention provides for a protein having a formula selected from the group consisting of: $R_1 - R_2$ and $R_1 - L - R_2$, wherein R_1 is a Fc protein, or a variant or fragment thereof, R_2 is an AGP-1 protein, or variant or fragment thereof, and L is a linker. The

invention provides for genetic or chemical linkages of the R1 and R2 moieties as described herein.

In one aspect, the present invention provides a Fc-AGP-1 fusion protein wherein Fc (or a variant, fragment or derivative thereof) is genetically fused to the N-terminus of an AGP-1 protein (or a variant, fragment or derivative thereof). In another aspect of the invention, an Fc portion may also be linked to the N-terminus of an AGP-1 protein (or a variant, fragment or derivative thereof) by a peptide or chemical linker as known in the art. As noted above and described in more detail below, the Fc-AGP-1 fusion protein has unexpected enhanced biological activity when compared to a soluble AGP-1 protein. Additional aspects of the present invention, therefore, include not only Fc-AGP-1 fusion protein compositions, but also nucleic acid sequences encoding such proteins, related vectors and host cells containing such vectors, both useful for producing fusion proteins of the present invention.

In a second aspect, the present invention provides for preparing the Fc-AGP-1 fusion protein. Such methods include recombinant DNA techniques for preparation of recombinant proteins. Furthermore, such aspects include methods of protein production and purification as well.

In another aspect, the present invention provides methods for treating proliferative disorders, such as cancer or cardiovascular diseases, viral infections and viral-induced diseases, and immune disorders by administration of Fc-AGP-1 fusion proteins.

In another aspect, the present invention also provides for related pharmaceutical compositions of the Fc-AGP-1 proteins, variants, fragments and derivatives thereof, for use in the above therapies.

Description of the Figures

Figure 1 (SEQ ID NO: 32) shows the amino acid sequence of the hinge, CH2 and CH3 regions of human IgGγ1.

Figure 2 (SEQ ID NOS: 33 and 34) shows the nucleotide and amino acid sequence of human AGP-1.

Figure 3 (SEQ ID NOS: 35 and 36) shows the nucleotide and amino acid sequence of Fc-huAGP-1 (95-281). Amino acids corresponding to OPG signal peptide are underlined. Amino acids corresponding to human AGP-1 (95-281) are bracketed.

Figure 4 (SEQ ID NOS: 37 and 38) shows the nucleotide and amino acid sequence of Fc-huAGP-1 (114-281). Amino acids corresponding to OPG signal peptide are underlined. Amino acids corresponding to human AGP-1 (114-281) are bracketed.

Figure 5 (SEQ ID NOS: 39 and 40) shows the nucleotide and amino acid sequence of Fc-muAGP-1 (99-291). Amino acids corresponding to OPG signal peptide are underlined. Amino acids corresponding to murine AGP-1 (99-291) are bracketed.

Figure 6 (SEQ ID NOS: 41 and 42) shows the nucleotide and amino acid sequence of Fc-muAGP-1 (120-291). Amino acids corresponding to OPG signal peptide are underlined. Amino acids corresponding to murine AGP-1(120-291) are bracketed.

Figure 7 shows the activity of soluble AGP-1 and Fc-AGP-1 fusion protein in inducing apoptosis in cultured Jurkat cells.

Detailed Description of the Invention

The present invention relates to Fc-AGP-1 fusion protein compositions, methods of preparation of such compositions and uses thereof. In particular, the present invention relates to the genetic or chemical fusion of the Fc region of immunoglobulins to the N-terminal portion of the AGP-1 protein. Unexpectedly, fusion of Fc at the N-terminus of the AGP-1 protein demonstrates advantages which are not seen in soluble AGP-1 protein. Surprisingly, the N-terminally modified Fc-AGP-1 protein provides unexpected increased biological activity. Accordingly, the Fc-AGP-1 fusion protein, and variants, fragments and derivatives thereof, as well as, related methods of use and preparation, are described in more detail below.

The term "Fc" refers to a molecule or sequence comprising the sequence of a non-antigen-binding portion of antibody, whether in monomeric or multimeric form. The original immunoglobulin source of an Fc is preferably of human origin and may be from any isotype, e.g., IgG, IgA, IgM, IgE or IgD. One method of preparation of an isolated Fc molecule involves digestion of an antibody with papain to separate antigen and non-antigen binding portions of the antibody. Another method of preparation of an isolated Fc molecules is production by recombinant DNA expression followed by purification of the Fc molecules so expressed. A full-length Fc consists of the following Ig heavy chain regions: CH1, CH2 and CH3 wherein the CH1 and CH2 regions are typically connected by a flexible hinge region. In one embodiment, an Fc has the amino acid sequence of IgG1 such as that shown in Figure 1. The terms "Fc protein, "Fc sequence", "Fc molecules, "Fc region" and "Fc portion" are taken to have the same meaning as "Fc".

The term "fragment" when used in association with Fc or AGP-1 polypeptides, or fusion polypeptides thereof, refers to a peptide or polypeptide that comprises less than the full length amino acid sequence of an Fc or AGP-1 polypeptide. Such a fragment may arise, for example, from a truncation at the amino terminus, a truncation at the carboxy terminus, and/or an internal deletion of a residue(s) from the amino acid sequence. AGP-1 or Fc fragments may result from alternative RNA splicing or from *in vivo* protease activity.

The term "variant" when used in association with Fc or AGP-1 polypeptides, or with fusion polypeptides thereof, refers to a polypeptide comprising an amino acid sequence which contain one or more amino acid sequence substitutions, deletions, and/or additions as compared to native Fc or AGP-1 polypeptide amino acid sequences. Variants may be naturally occurring or artificially constructed. Variants of the invention may be prepared from the corresponding nucleic acid molecules encoding said variants, which have a DNA sequence that varies accordingly from the DNA sequences for native Fc or AGP-1 polypeptides.

The term "derivative" when used in association with Fc or AGP-1 polypeptides, or with fusion polypeptides thereof, refers to Fc or AGP-1 polypeptide variants or fragments thereof, that have been chemically modified, as for example, by covalent attachment of one or more polymers, including, but limited to, water soluble polymers, N-linked or O-linked carbohydrates, sugars, phosphates, and/or other such molecules. The derivatives are modified in a manner that is different from native Fc or AGP-1, either in the type or location of the molecules attached to the polypeptide. Derivatives further

includes deletion of one or more chemical groups naturally attached to an Fc or AGP-1 polypeptide.

The term "fusion" refers to joining of different peptide or protein segments by genetic or chemical methods wherein the joined ends of the peptide or protein segments may be directly adjacent to each other or may be separated by linker or spacer moieties such as amino acid residues or other linking groups.

Compositions

The invention provides for FcAGP-1 fusion polypeptides and compositions thereof. Fusions of an Fc region to an AGP-1 polypeptide are advantageously made at the amino terminus of AGP-1, that is, the carboxy terminus of an Fc region is fused to the amino terminus of AGP-1. These fusion proteins (and nucleic acids encoding same) are designated herein as FcAGP-1. However, it is also contemplated that, in certain instances, it may be desirable to fuse the carboxy terminus of AGP-1 to the amino terminus of an Fc region. The fusion proteins (and nucleic acids encoding same) are designated herein as AGP-1Fc.

An Fc, or a variant, fragment or derivative thereof, may be from an Ig class. In one embodiment, an Fc is from the IgG class, such as IgG1, IgG2, IgG3, and IgG4. In another embodiment, an Fc is from IgG1. An Fc may also comprise amino acid residues represented by a combination of any two or more of the Ig classes, such as residues from IgG1 and IgG2, or from IgG1, IgG2 and IgG3, and so forth. In one embodiment, an Fc region of an Fc-AGP-1 fusion protein has the sequence as set forth in Figure 1 (SEQ ID NO: 32) comprising hinge CH2 and CH3 regions of human IgG1. (see Ellison *et al.*, Nucleic Acids Res. 10, 4071-4079 (1982).

In addition to naturally occurring variations in Fc regions, Fc variants, fragments and derivatives

may contain non-naturally occurring changes in Fc which are constructed by, for example, introducing substitutions, additions, insertions or deletions of residues or sequences in a native or naturally occurring Fc, or by modifying the Fc portion by chemical modification and the like. In general, Fc variants, fragments and derivatives are prepared such that the increased circulating half-life of Fc fusions to AGP-1 is largely retained.

Also provided by the invention are Fc variants with conservative amino acid substitutions. The term "conservative amino acid substitution" refers to a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. For example, a conservative substitution results from the replacement of a non-polar residue in a polypeptide with any other non-polar residue. General rules for conservative amino acid substitutions are set forth in Table I.

Table I

Conservative Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val,Leu,Ile	Val
Arg	Lys,Gln,Asn	Lys
Asn	Gln,His,Lys,Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro,Ala	Ala
His	Asn,Gln,Lys,Arg	Arg
Ile	Leu,Val,Met,Ala, Phe,Norleucine	Leu

Leu	Norleucine,Ile, Val,Met,Ala,Phe	Ile
Lys	Arg,Gln,Asn	Arg
Met	Leu,Phe,Ile	Leu
Phe	Leu,Val,Ile,Ala, Tyr	Leu
Pro	Ala	Ala
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr,Phe	Tyr
Tyr	Trp,Phe,Thr,Ser	Phe
Val	Ile,Met,Leu,Phe, Ala,Norleucine	Leu

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties. Conservative modifications to the amino acid sequence (and the corresponding modifications to the encoding nucleotides) are expected to produce Fc molecules (and FcAGP-1 fusion proteins) having functional and chemical characteristics similar to those of unmodified Fc and FcAGP-1 proteins.

In addition to the substitutions set forth in Table I, any native residue in an Fc region (or in an FcAGP-1 fusion protein) may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (Cunningham et al. Science 244, 1081-1085 (1989)).

Substantial modifications in the functional and/or chemical characteristics of an Fc molecule (and an FcAGP-1 fusion protein) may be accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for

example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues may be divided into groups based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr;
3) acidic: Asp, Glu;
4) basic: Asn, Gln, His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of an Fc or AGP-1 molecule that are homologous with non-human Fc or AGP-1, or into the non-homologous regions of the molecule.

Cysteine residues in Fc molecules can be deleted or replaced with other amino acids to prevent formation of disulfide crosslinks. In particular, a cysteine residue at position 5 of Figure 1 (SEQ. ID. NO. 32) may be substituted with one or more amino acids, such as alanine or serine. Alternatively, the cysteine residue at position 5 could be deleted.

An Fc fragment may be prepared by deletion of one or more amino acids at any of positions 1, 2, 3, 4 and 5 as shown in Figure 1 (SEQ ID NO. 32). In one embodiment, the amino acid residues at positions 1-5 inclusive are deleted. Substitutions at these positions can also be made and are with in the scope of this invention.

Fc variants may also be made which show reduced binding to Fc receptors which trigger effector functions such as antibody dependent cellular

cytotoxicity (ADCC) and activation of complement. Such variants may include leucine at position 20 deleted or substituted with a glutamine residue, glutamate at position 103 deleted or substituted with an alanine residue, and lysines at positions 105 and 107 deleted or substituted with alanine residues (following the numbering as set forth in Figure 1). One or more of such substitutions are contemplated.

In one embodiment, Fc variants will exhibit stronger binding to the FcRn receptor ("salvage receptor") and a longer circulating half-life compared to native Fc such as that shown in Figure 1. Example of such variants include amino acid substitutions at one or more of residues 33, 35-42, 59, 72, 75, 77, 95-98, 101, 172-174, 215 and 220-223, wherein the substitution(s) confer tighter binding of an Fc variant to the FcRn receptor.

Other Fc variants include one or more tyrosine residues replaced with, for example, phenyalanine residues. In addition, other variant amino acid insertions, deletions and/or substitutions are also contemplated and are within the scope of the present invention. Examples include Fc variants disclosed in WO96/32478 and WO97/34630 hereby incorporated by reference. Furthermore, alterations may be in the form of altered amino acids, such as peptidomimetics or D-amino acids.

The Fc protein may be also linked to the AGP-1 proteins of the Fc-AGP-1 protein by "linker" moieties whether chemical or amino acids of varying lengths. Such chemical linkers are well known in the art. Amino acid linker sequences can include but are not limited to:

(a) ala-ala-ala;

(b) ala-ala-ala-ala;

(c) ala-ala-ala-ala-ala;

(d) gly-gly;
(e) gly-gly-gly;
(f) gly-gly-gly-gly-gly;
(g) gly-gly-gly-gly-gly-gly-gly;
(h) gly-pro-gly;
(i) gly-gly-pro-gly-gly; and
(j) any combination of subparts (a) through (i).

AGP-1 variants, fragments and derivatives are also provided by the invention and are generally as described hereinabove for Fc molecules, with the exception of the specific locations of the modified amino acid residues. In a preferred embodiment, AGP-1 is a soluble form of AGP-1 which is not membrane-bound and lacks a functional transmembrane domain. As an example, soluble AGP-1 may comprise an extracellular domain and lack sequences for cytoplasmic and transmembrane domains. The full-length human AGP-1 extracellular domain encompasses about residues 39-281 inclusive using the numbering system as set forth in Figure 2. Soluble human AGP-1 may also encompass fragments of a full-length extracellular domain which function to bind receptor or to induce apoptosis in an assay such as that described in Example 4 below. Soluble AGP-1 fragments comprise the amino acid sequence X-281 wherein X is any residue from 95 to 114 inclusive using the numbering system of as set forth in Figure 2. Other soluble AGP-1 fragments encompass residues 115-281, 116-281, 117-281, 118-281 and 119-281 inclusive. Variants and derivatives of the AGP-1 fragments described herein are also encompassed by the invention.

Nucleic acid molecules

Nucleic acid molecules encoding Fc-AGP-1 proteins, or variants, fragments or derivatives thereof, are provided for by the invention. Nucleic acid molecules of the invention may be produced using site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired mutations. See Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory Press, Cold Springs Harbor, N.Y. (1989)), and Ausubel et al. (Current Protocols in Molecular Biology, Wiley and Sons, N.Y. (1994)), for descriptions of mutagenesis techniques. Chemical synthesis using methods described by Engels et al. (Angew. Chem. Intl. Ed. 28, 716-734 (1989)), may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have been altered for optimal expression of an Fc-AGP-1 polypeptide in a given host cell. Particular codon alterations will depend upon the Fc-AGP-1 polypeptide(s) and host cell(s) selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host cell. Computer algorithms which incorporate codon frequency tables such as "Ecohigh. Cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI. Other useful codon frequency tables include "Celegans_high.cod", "Celegans_low.cod", "Drosophila_high.cod", "Human_high.cod", "Maize_high.cod", and "Yeast_high.cod".

In other embodiments, nucleic acid molecules encode Fc-AGP-1 variants with conservative amino acid

substitutions as defined hereinabove. Also provided for are Fc or AGP-1 variants comprising an addition and/or a deletion of one or more N-linked or O-linked glycosylation sites, or Fc or AGP-1 polypeptide fragments as described above. In addition, nucleic acid molecules may encode any combination of Fc and/or AGP-1 variants, fragments, and fusion polypeptides described herein.

Vectors and Host cells

A nucleic acid molecule encoding an Fc-AGP-1 fusion protein is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding an Fc-AGP-1 protein may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether an Fc-AGP-1 protein is to be post-translationally modified (e.g, glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian host cells are preferable.

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotides: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a leader sequence for secretion, a ribosome binding site,

a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element.

Flanking sequences may be homologous (i.e., from the same species and/or strain as the host cell), heterologous (i.e, from a species other than the host cell species or strain), hybrid (i.e., a combination of flanking sequences from more than one source), or synthetic, or native sequences which normally function to regulate AGP-1 and/or Fc protein expression. As such, the source of flanking sequences may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequences is functional in, and can be activated by, the host cell machinery.

A leader, or signal, sequence may be used to direct an Fc-AGP-1 polypeptide out of the host cell. Typically, the signal sequence is positioned in the coding region of the Fc-AGP-1 nucleic acid molecule, or directly at the 5' end of the Fc-AGP-1 polypeptide coding region. Many signal sequences have been identified, and any of them that are functional in the selected host cell may be used in conjunction with nucleic acid sequences encoding Fc-AGP-1 proteins. Therefore, a signal sequence may be homologous (naturally occurring) or heterologous to the AGP-1 or Fc gene or cDNA, Additionally, a signal sequence may be chemically synthesized using methods set forth above. In most cases, secretion of an Fc-AGP-1 polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the fusion polypeptide.

The signal sequence may be a component of the vector, or it may be a part of Fc-AGP-1 DNA that is inserted into the vector. Native AGP-1 DNA encodes a signal sequence at the amino terminus of the protein

that is cleaved during post-translational processing of the molecule to form the mature protein (see Figure 2). Included within the scope of this invention are AGP-1 nucleotides with the native signal sequence as well as AGP-1 nucleotides wherein the native signal sequence is deleted and replaced with a heterologous signal sequence. A heterologous signal sequence selected should be one that is recognized and processed, i.e., cleaved by a signal peptidase, by the host cell. In one embodiment, a heterologous signal sequence is the OPG signal sequence as described in WO97/23614. For prokaryotic host cells that do not recognize and process the native AGP-1 signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, the native AGP-1 signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15b (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional possible vectors include, but are not limited to, cosmids, plasmids or modified viruses, but the vector system must be compatible with the

selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO™ TA Cloning® Kit, PCR2.1® plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian , yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, or other known techniques. After the vector has been constructed and a nucleic acid molecule encoding an AGP-1 polypeptide has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). The host cell, when cultured under appropriate conditions, synthesizes an AGP-1 polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). Selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

Suitable host cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC #CCL61 and Urlaub et al., Proc. Natl. Acad. Sci. USA 77, 4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC #CRL1573), or 3T3 cells

(ATCC #CRL1658). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 and COS-7 cell lines (ATCC #CRL1651), and the CV-1 cell line (ATCC #CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines. Each of these cell lines is known by and available to those skilled in the art.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, DH5a, DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerivisae*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts *et al.* (*Biotechniques*, 14, 810-817 (1993)),

Lucklow (*Curr. Opin. Biotechnol.*, 4, 564-572 (1993))and Lucklow *et al.* (*J. Virol.*, 67, 4566-4579 (1993)). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

Transformation or transfection of an expression vector for an AGP-1 polypeptide into a selected host cell may be accomplished by well known methods including methods such as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

Polypeptide Production

Host cells comprising an AGP-1 expression vector (*i.e.*, transformed or transfected) may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells are for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum and/or growth factors as required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary (Gibco Life Technologies, Gaithersburg, MD).

Typically, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with

which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin; where the selectable marker element is ampicillin resistance, the compound added to the culture medium will be ampicillin.

The amount of an AGP-1 polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

Where an AGP-1 polypeptide is prepared without a tag attached, and no antibodies are available, other well known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined to achieve increased purity.

If an AGP-1 polypeptide is produced intracellularly, the intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

If an AGP-1 polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often

bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. An AGP-1 polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate an AGP-1 polypeptide, isolation may be accomplished using standard methods such as those set forth below and in Marston et al. (Meth. Enz., 182, 264-275 (1990)).

In some cases, an AGP-1 polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages, can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/dithiane DTT, and 2-

mercaptoethanol(bME)/dithio-b(ME). In many instances, a cosolvent may be used or may be needed to increase the efficiency of the refolding and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

Derivatives

The present Fc-AGP-1 fusion proteins, and variants and fragments thereof, are derivatized by the attachment of one or more chemical moieties to an Fc-AGP-1 fusion protein moiety. These chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular subcutaneous, intravenous, oral, nasal, pulmonary, topical or other routes of administration as discussed below. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. See, U.S. Patent No. 4,179,337. For a review, see Abuchowski *et al.*, in Enzymes as Drugs. (J. S. Holcerberg and J. Roberts, eds. pp. 367-383 (1981)); Francis *et al.*, supra.

The chemical moieties suitable for such derivatization may be selected from among various water soluble polymers. The polymer selected should be water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically,

and if so, the desired dosage, circulation time, resistance to proteolysis, and other considerations. For the present proteins, the effectiveness of the derivatization may be ascertained by administering the derivative, in the desired form (*i.e.*, by osmotic pump, or, more preferably, by injection or infusion, or, further formulated for oral, pulmonary or nasal delivery, for example), and observing biological effects as described herein.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohol. Polyethylene glycol propionaldenhyde may have advantages in manufacturing due to its stability in water. Also, succinate and styrene may also be used.

Fc-AGP-1 fusion proteins, variants and fragments may also be derivatized by attaching polyaminoacids or branch point amino acids to the Fc or AGP-1 protein (or variant or fragment) moiety. For example, the polyaminoacid may be an additional carrier protein which serves to further increase the circulation half life of an Fc-AGP-1 fusion protein in addition to the advantages achieved via the Fc-AGP-1 fusion protein above. For the present therapeutic or cosmetic purpose of the present invention, such polyaminoacids should be those which have or do not create neutralizing antigenic response, or other adverse responses. Such polyaminoacids may be selected

from the group consisting of serum album (such as human serum albumin), or other polyaminoacids, *e.g.* lysines. As indicated below, the location of attachment of the polyaminoacid may be at the N-terminus of the Fc-AGP-1 protein moiety, or C-terminus, or other places in between, and also may be connected by a chemical "linker" moiety to the Fc-AGP-1 protein.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 2 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (*e.g.*, the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The number of polymer molecules so attached may vary, and one skilled in the art will be able to ascertain the effect on function. One may mono-derivatize, or may provide for a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (*e.g.*, polymers, such as different weights of polyethylene glycols). The proportion of polymer molecules to protein (or peptide) molecules will vary, as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted protein or polymer) will be determined by factors such as the desired degree of derivatization (*e.g.*, mono, di-, tri-, etc.), the molecular weight of

the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

The chemical moieties should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art. *E.g.*, EP 0401384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, Exp. Hematol. 20, 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residue. Those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding is desired.

One may specifically desire N-terminally chemically modified Fc-AGP-1 fusion protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of

obtaining the N-terminally pegylated preparation (*i.e.*, separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. For example, one may selectively N-terminally pegylate the protein by performing the reaction at a pH which allows one to take advantage of the pK_a differences between the e-amino group of the lysine residues and that of the a-amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a water soluble polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs. Using reductive alkylation, the water soluble polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

An N-terminally monopegylated derivative is preferred for ease in production of a therapeutic. N-terminal pegylation ensures a homogenous product as characterization of the product is simplified relative to di-, tri- or other multi-pegylated products. The use of the above reductive alkylation process for

preparation of an N-terminal product is preferred for ease in commercial manufacturing.

Uses of the Polypeptide

AGP-1 fusion proteins may be used for the treatment of proliferative disorders wherein cells are undergoing excessive proliferation. For example, an AGP-1 fusion protein may be used as an anti-tumor to treat patients suffering from a variety of cancers, such as breast cancer, prostate cancer, lung cancer, and colon cancer. Viral infections and viral-induced diseases, such as hepatitis and AIDS, may also be treated with the proteins of the invention. Cardiovascular diseases such as arteriosclerosis which characterized by excessive proliferation of vascular smooth muscle cells may also be treated. AGP-1 fusion proteins may also be used to suppress T-lymphocyte mediated immune responses that occur in autoimmune disorders and in rejection of transplanted tissues It is desirable that that present proteins are expected to have prolonged in vivo half-lives and circulation times that will allow for lower dosages, less frequent administration, and enhanced efficacy compared to an unfused AGP-1 protein.

Pharmaceutical Compositions

The present invention also provides for pharmaceutical compositions of the Fc-AGP-1 fusion proteins, variants, fragments and derivatives. Such pharmaceutical compositions may be for administration for injection, or for oral, pulmonary, nasal, transdermal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of protein or derivative products of the invention together with pharmaceutically acceptable

diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. An effective or a therapeutically effective amount of an FcAGP-1 fusion protein is an amount sufficient to induce apoptosis in a target cell, wherein apoptosis is evaluated by assays known in the art.

Such compositions include diluents of various buffer content (*e.g.*, Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (*e.g.*, Tween 80, Polysorbate 80), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), preservatives (*e.g.*, Thimersol, benzyl alcohol) and bulking substances (*e.g.*, lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, *e.g.*, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

Contemplated for use herein are oral solid dosage forms, which are described generally in Remington's Pharmaceutical Sciences, 18th Ed. 1990 (Mack Publishing Co. Easton PA 18042) at Chapter 89, which is herein incorporated by reference. Solid dosage forms include tablets, capsules, pills, troches

or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (*e.g.*, U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given by Marshall, K. In: *Modern Pharmaceutics* Edited by G. S. Banker and C. T. Rhodes Chapter 10, 1979, herein incorporated by reference. In general, the formulation will include the Fc-AGP-1 fusion protein (or analog or derivative), and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

Also specifically contemplated are oral dosage forms of the above derivatized proteins. FcAGP-1 fusion protein may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the protein (or peptide) molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the protein and increase in circulation time in the body. Examples of such moieties include: Polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. Abuchowski and Davis, Soluble Polymer-Enzyme Adducts. In: "Enzymes as Drugs", Hocenberg and RAGP-1erts, eds., Wiley-Interscience, New York, NY, (1981), pp 367-383; Newmark, *et al.*, J. Appl. Biochem. 4: 185-189 (1982). Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for

pharmaceutical usage, as indicated above, are polyethylene glycol moieties.

To ensure resistance to degradation in the stomach following oral administration, a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings for oral formulations are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

The therapeutic can be included in the formulation as fine multiparticulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

One may dilute or increase the volume of an FcAGP-1 composition with an inert material. These diluents could include carbohydrates, especially mannitol, α-lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrates include but are not limited to starch including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose,

ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An antifrictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the therapeutic into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl

sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethomium chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

Additives which potentially enhance uptake of the protein (or derivative) are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

Controlled release formulation may be desirable. The drug could be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms *e.g.*, gums. Slowly degenerating matrices may also be incorporated into the formulation, *e.g.*, alginates, polysaccahrides. Another form of a controlled release of this therapeutic is by a method based on the Oros therapeutic system (Alza Corp.), *i.e.*, the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

Other coatings may be used for the formulation. These include a variety of sugars which could be applied in a coating pan. The therapeutic agent could also be given in a film coated tablet and the materials used in this instance are divided into 2 groups. The first are the nonenteric materials and include methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium

carboxy-methyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

A mix of materials might be used to provide the optimum film coating. Film coating may be carried out in a pan coater or in a fluidized bed or by compression coating.

Also contemplated herein is pulmonary delivery of the present protein (or derivatives thereof). The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. (Other reports of this include Adjei *et al.*, Pharmaceutical Research 7: 565-569 (1990); Adjei *et al.*, International Journal of Pharmaceutics 63: 135-144 (1990)(leuprolide acetate); Braquet *et al.*, Journal of Cardiovascular Pharmacology 13 (suppl. 5): s.143-146 (1989)(endothelin-1);Hubbard *et al.*, Annals of Internal Medicine 3: 206-212 (1989)(α1-antitrypsin); Smith *et al.*, J. Clin. Invest. 84: 1145-1146 (1989)(α1-proteinase); Oswein *et al.*, "Aerosolization of Proteins", Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, 1990 (recombinant human growth hormone); Debs *et al.*, The Journal of Immunology 140: 3482-3488 (1988)(interferon γ and tumor necrosis factor α) and U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor).

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of formulations suitable for the dispensing of protein (or analog or derivative). Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to diluents, adjuvants and/or carriers useful in therapy.

The protein (or derivative) should most advantageously be prepared in particulate form with an average particle size of less than 10 μm (or microns), most preferably 0.5 to 5 μm, for most effective delivery to the distal lung.

Carriers include carbohydrates such as trehalose, mannitol, xylitol, sucrose, lactose, and sorbitol. Other ingredients for use in formulations may include DPPC, DOPE, DSPC and DOPC. Natural or synthetic surfactants may be used. Polyethylene glycol may be used (even apart from its use in derivatizing the protein or analog). Dextrans, such as cyclodextran, may be used. Bile salts and other related enhancers may be used. Cellulose and cellulose derivatives may be used. Amino acids may be used, such as use in a buffer formulation.

Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

Nasal delivery of the protein (or analog or derivative) is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran. Delivery via transport across other mucus membranes is also contemplated.

Dosage

One skilled in the art will be able to ascertain effective dosages by administration and observing the desired therapeutic effect. Due to the modification of an AGP-1 protein by fusion to an Fc, the present invention provides unexpected protein protection from degradation, and increases circulation time and stability, when compared to a soluble AGP-1 protein. One skilled in the art, therefore, will be able to ascertain from these changes that an effective dosage may require lower doses or less frequent dosing.

Preferably, the formulation of the molecule will be such that between about .10 µg/kg and 10 mg/kg will yield the desired therapeutic effect. The frequency of administration may be readily determined by one skilled in the art. The effective dosages may be determined using diagnostic tools over time. For example, a diagnostic for measuring the amount of AGP-1 protein or Fc-AGP-1 fusion protein in the blood (or plasma or serum) may first be used to determine endogenous levels of protein. Such diagnostic tools may be in the form of an antibody assay, such as an antibody sandwich assay. The amount of endogenous AGP-1 protein is quantified initially, and a baseline is determined. The therapeutic dosages are determined as the quantification of endogenous and exogenous AGP-1

protein or Fc-AGP-1 fusion protein (that is, protein, variant, fragment or derivative found within the body, either self-produced or administered) is continued over the course of therapy. The dosages may therefore vary over the course of therapy, with a relatively high dosage being used initially, until therapeutic benefit is seen, and lower dosages used to maintain the therapeutic benefits.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Production of AGP-1 Fusion Proteins

The fusion proteins Fc-huAGP-1 (95-281), Fc-huAGP-1 (114-281), Fc-muAGP-1 (99-291) and Fc-muAGP-1 (120-291) were constructed by the following procedures.

The human IgGγ1 Fc region was PCR amplified with the following set of oligonucleotide primers:

5' TCT CCA AGC TTG AGC CCA AAT CTT GTG ACA AAA C 3' (SEQ ID NO. 1)

5' TCT CCC TTA AGT TTA CCC GGA GAC AGG GAG AG 3' (SEQ ID NO. 2)

PCR reaction was carried out in a volume of 50 µl with 1 unit of vent DNA polymerase (New England Biolabs) in 20 mM Tris-HCl pH8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton-X100, 10 µM of each dNTP, 1µM of each primer and 10 ng of RANKFc/pCEPP4 template (Hsu et al, Proc. Natl. Acad. Sci. USA 96, 3540-3545 (1999)). Reactions were performed in 94°C for 30 sec., 55°C for 30 sec., and

72°C for 1 min, for a total of 16 cycles. The PCR fragment was isolated by electrophoresis through 1% agarose and purification by the Geneclean procedure (Bio 101, Inc.). The PCR fragment creates a HindIII restriction site at 5' end and a AflII restriction site at 3' end. The HindIII-AflII digested PCR fragment was then subcloned into the pCEP4 vector (Invitrogen) to create Fc/pCEP4.

A murine OPG signal peptide having the following amino acid sequence:

MNKWLCCALLVLLDIIEWTTQ (SEQ ID NO. 3)

was created by annealing the following set of oligonucleotides:

5' CTA GCA CCA TGA ACA AGT GGC TGT GCT GCG CAC TCC TGG TGC TCC TGG ACA TCA TTG AAT GGA CAA CCC AGA 3' (SEQ ID NO. 4)

5' AGC TTC TGG GTT GTC CAT TCA ATG ATG TCC AGG AGC ACC AGG AGT GCG CAG CAC AGC CAC TTG TTC ATG GTG 3' (SEQ ID NO. 5)

The annealing was carried out by heating 10 μM of each oligonucleotide in a total volume of 20 μl at 94°C for 5 minutes, and then cooling gradually to room temperature. The annealed oligonucleotides, creating NheI restriction site overhang at 5' end and HindIII restriction site overhang at 3' end, were cloned into Fc-pCEP4 vector inframe N-terminal to the human IgGγ1 Fc region to generate SO-Fc/pCEP4 vector. A linker which encodes two irrelevant amino acids (QK) was introduced between the OPG signal peptide and human IgGγ1 Fc. An extra irrelevant amino acid (K) was introduced by the HindIII site between the OPG signal peptide and human IgGγ1 Fc.

The baculovirus expression system chosen to express nucleic acid sequences encoding Fc-AGP-1 fusion proteins is based on the BAC-TO-BACK expression system (Life Technologies, Gaithersburg, MD) with the following modifications.

The major capsid protein promoter sequence (GenBank Acc. No. M22978) was PCR amplified from the Bac-N-Blue™ linear AcMNPV DNA purchased from Invitrogen (Carlsbad, CA) with the following primers:

5' ATT ATT GAT ATC GCA TGC TTG TTC GCC ATC GTG GAA TC (SEQ ID NO. 6)
5' AAT CCG GAA TAT TGT TGC CGT TAT AAA TAT GGA C (SEQ ID NO. 7)

The N-terminal coding sequence of the first 12 codons of the polyhedrin gene with the methionine start codon mutated from ATG to ATT and the following MCS were PCR amplified from the vector pBlueBac4 DNA (Invitrogen) with the following primers:

5' AAC GGC AAC AAT ATT CCG GAT TAT TCA TAC CGT CC (SEQ ID NO. 8)
5' ACT TCA AGG AGA ATT TCC (SEQ ID NO. 9)

The resulting two fragments share overlapping sequence and a second round of PCR was performed to fuse them together. The polyhedrin promoter and the multiple cloning sites (MCS) between the SnaBI and HindIII site present in pFastBac1 was then replaced with the EcoRV and HindIII digested fragment containing capsid promoter and the new MCS. The resulting vector is named pFC. To further modify the MCS, the following

pair of primers were annealed together and ligated to the pFC vector digested with NheI and HindIII.

5' CTA GCT CTA GAC ATA TGG AAT TCC TGC AGC AGC TGG TAC CTC GAG GATCCA AGC TTG TCG ACT(SEQ ID NO. 10)
5' AGC TAG TCG ACA AGC TTG GAT CCT CGA GGT ACC AGC TGC TGC AGG AAT TCC ATA TGT CTA GAG (SEQ ID NO. 11)

The resulting vector is named pFC1 and is used as the donor vector for all baculovirus expression work described here.

To generate Fc-AGP-1 sequences in a baculovirus expression vector, the SO-Fc/pCEP4 was first used as template for PCR amplification of OPG signal peptide followed by human IgGγ1 Fc region with the following primers:

5' GGG CGT GCT AGC CAC CAT GAA CAA GTG GCT GTG CTG C 3' (SEQ ID NO. 12)
5' AGC TCC TTC TGC AGG TGG AAC AGC TGT TTA CCC GGA GAC AGG GAG 3' (SEQ ID NO. 13)

PCR reactions were carried out under conditions similar to those described above. The PCR fragment creates NheI restriction site at 5' end and PstI restriction site at 3' end, and was subsequently cloned into pFC1 vector to create SO-Fc/pFC1.

Human AGP-1(114-281) was PCR amplified from human AGP-1 and human IgGγ1 cDNA templates by the following overlapping set of primers to generate PCR fusion fragments:

5' CTC CGG GTA AAG TGA GAG AAA GAG GTC CTC AG 3' (SEQ ID NO. 14)

5' TTC TCT CAC TTT ACC CGG AGA CAG GGA G 3' (SEQ ID NO. 15)
5' CTT CTT CCT CTA CAG CAA GC 3' (SEQ ID NO. 16)
5' GTT ATT GCT CAG CGG TGG CA 3' (SEQ ID NO. 17)

Human AGP-1(95-281) was PCR amplified from human AGP-1 and human IgGγ1 cDNA templates by the following overlapping set of primers to generate PCR fusion fragments:

5' CCG GGT AAA ACT TCT GAG GAA ACC ATT TCT AC 3' (SEQ ID NO. 18)
5' TCC TCA GAA GTT TTA CCC GGA GAC AGG GAG AG 3' (SEQ ID NO. 19)
5' CTT CTT CCT CTA CAG CAA GC 3' (SEQ ID NO. 20)
5' GTT ATT GCT CAG CGG TGG CA 3' (SEQ ID NO. 21)

Murine AGP-1(120-291) was PCR amplified from murine AGP-1 and human IgG g1 cDNA templates by the following overlapping set of primers to generate PCR fusion fragments:

5' CCG GGT AAA GGT GGA AGA CCT CAG AAA GTG 3' (SEQ ID NO. 22)
5' GAG GTC TTC CAC CTT TAC CCG GAG ACA GGG AG 3' (SEQ ID NO. 23)
5' CTT CTT CCT CTA CAG CAA GC 3' (SEQ ID NO. 24)
5' GTT ATT GCT CAG CGG TGG CA 3' (SEQ ID NO. 25)

Murine AGP-1(99-291) was PCR amplified from human AGP-1 and human IgG g1 cDNA templates by the following overlapping set of primers to generate PCR fusion fragments:

5' CTC CGG GTA AAA CCT TTC AGG ACA CCA TTT CTA C 3' (SEQ ID NO. 26)

5' CCT GAA AGG TTT TAC CCG GAG ACA GGG AG 3' (SEQ ID NO. 27)

5' CTT CTT CCT CTA CAG CAA GC 3' (SEQ ID NO. 28)

5' GTT ATT GCT CAG CGG TGG CA 3' (SEQ ID NO. 29)

The PCR was carried out in similar conditions as described above. The resulting PCR fusion fragments were digested with NsiI and XhoI restriction enzymes and cloned in frame C-terminal to the human IgGg1 Fc region in SO-Fc/pFC1 at NsiI and XhoI sites. The final pFC1 constructs expressing Fc-AGP-1 fusion proteins were subject to sequencing analysis.

Transformation of the donor plasmids, selection of the recombinant bacmids and isolation of the recombinant bacmid DNAs followed the protocols provided by the manufacturer (Life Technologies). The recombinant bacmid DNAs described above were transfected into insect SF-9 cells by standard calcium chloride precipitation method to generate recombinant viruses using Grace's insect cell medium with supplements (Invitrogen) and 10 % fetal bovine serum. The viruses were subsequently amplified using the same cell line and medium. The final titer of the viruses was estimated to be around 10^7 (pfu/ml) based on previous experiences. To produce Fc-AGP-1 fusion proteins, shake flask expression was set up to infect insect Hi-Five cells at a density of 2 x 10^6 cells/ml grown in serum-free Ex-CELL405 medium from JRH Biosciences (Lenexa, KS). The amount of virus preparation used was about 1/10 of the total cell culture volume. Time course samples were collected and analyzed by Western blot analysis in a pilot experiment and the harvest time was determined to be at 50-55 hours post-infection. Some product degradation was observed at later hours and when more cells (> 10 %) started to show signs of death. The harvested conditioned medium was filter-

sterilized immediately and kept at -80°C until purification step.

EXAMPLE 2
Purification of AGP-1 Fusion Proteins

Fc-AGP-1 fusion proteins were purified using Pharmacia Protein A Sepharose. The resin was equilibrated with TBS containing 20mM Tris pH7.0 and 150mM NaCl before applying the media. Complete protease inhibitor cocktail (Boehringer-Mannheim) was added to the media according to the manufacturer's instructions. The media was loaded, the column washed with TBS, and protein was eluted using Gentle Elution buffer (Pierce, Rockford, IL). Protein containing fractions were pooled and submitted for in vitro analysis.

EXAMPLE 3
Production of AGP-1 Protein

PCR amplification employing the following primer pair and human AGP-1 cDNA template are used to generate various forms of human AGP-1.

5' ATT TGA TTC TAG AAG GAG GAA TAA CAT ATG GTT CGT GAA CGT GGT CCA CAG CGT GTA GCA 3' (SEQ ID NO. 30)

5' TAT CCG CGG ATC CTC GAG TTA GCC AAC TAA AAA GGC CCC GAA 3' (SEQ ID NO. 31)

One primer introduces unique XbaI and NdeI restrictions sites, an initiating Mmthionine codon, and optimized codons for the amino terminal protein of the

gene. The other primer of the pair introduces a TAA stop codon, and a unique XhoI site following the carboxyl terminus of the gene. PCR and thermocycling was performed using standard recombinant DNA methodology. The PCR products were purified, restriction digested and inserted into the unique XbaI and XhoI sites of the vector pAMG21 (ATCC accession no. 98113), and transformed into the prototrophic E.coli strain 2596. The resulting construct pAMG21-huAGP-1 (114-281) was engineered to encode human AGP-1 from amino acids 114-281 (see Figure 2). After transformation, clones were selected, plasmid DNA was isolated and the sequence of the AGP-1 gene insert was confirmed.

Human AGP-1(114-281) was purified as follows. 40.5g of E. coli cell paste was homogenized in 20mM Tris, 10mM EDTA, pH7.5 with Complete protease inhibitors in a total volume of 250mL. Cells were lysed with two passes in a microfluidizer at70psi and then centrifuged at 14,000 rpm in a JA14 rotor for 60min. The supernatant was filtered through 0.45µm and 0.22µm pore size filters. Aliquots were stored at -20°C. One 10mL aliquot was thawed and centrifuged. The supernatant was diluted 1:2 with 20mM Tris pH 7.5, adjusted to pH 7.5 then filtered through a 0.45µm filter. The sample was then applied to an 5mL SP Hi Trap column which had been equilibrated with the above buffer. The protein was eluted using a gradient from 0-0.5M NaCl in 20mM Tris pH 7.5. AGP-1 containing fractions were concentrated and then diafiltered into PBS pH6.2 using a centriprep 30. The concentrated fraction was estimated to be 90% pure by SDS-PAGE stained with Coomassie Blue.

EXAMPLE 4

Biological Activity of AGP-1 and AGP-1 Fusion Proteins

Jurkat cells (ATCC No. ___) were maintained in RPMI medium 1640 containing 10% fetal calf serum, 100 mg/ml penicillin G, and 100 mg/ml streptomycin (GIBCO). To study apoptotsis induced by FcAGP-1 recombinant protein, 250 ml of Jurkat cells (5 x 105 cells/ml) were seeded to each well of a 96 well plate. Cells were incubated in 5% CO_2 at 37°C with indicated concentrations of hu AGP-1 (114-281), huFcAGP-1 (114-281), or human IgG (Sigma) for 24 hours. Alamar Blue (Biosource Inc.) was added to each well in an amount equal to 10% of the culture volume. Cells were incubated for another 8 hours. Fluorescence was measured with excitation wavelength at 530nm and emission wavelength at 590nm in Bio-TEK FL500 Fluorescence Plate Reader (Bio-TEK Insturments Inc.). Each experiment was performed in duplicates. The results shown in Figure 7 indicate enhanced apoptosis activity of FcAGP-1 compared to soluble AGP-1.

* * *

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

WHAT IS CLAIMED IS:

1. A protein having a formula selected from the group consisting of: $R_1 - R_2$ and $R_1 - L - R_2$, wherein R_1 is a Fc protein, or variant or fragment thereof, R_2 is an AGP-1 protein, or variant or fragment thereof, and L is a linker.

2. The protein according to claim 1, where in the Fc protein is selected from the group consisting of:

(a) the Fc amino acid sequences as set forth in Figure 1;

(b) the amino acid sequence of subpart (a) having a different amino acid substituted or deleted in one or more of the following positions (using the numbering according to Figure 1):

(i) one or more cysteine residues;

(ii) one or more tyrosine residues;

(iii) cysteine at position 5 deleted or substituted with an alanine;

(iv) leucine at position 20 deleted or substituted with glutamine;

(v) glutamic acid at position 103 deleted or substituted with an alanine;

(vi) lysine at position 105 deleted or substituted with an alanine;

(vii) lysine at position 107 deleted or substituted with an alanine;

(viii) deletion or substitution of one or more of the amino acids at positions 1, 2, 3, 4, and 5;

(ix) one or more residues substituted or deleted to ablate the Fc receptor binding site;

(x) one or more residues substituted or deleted to ablate the complement (C1q) binding site; and

(xi) a combination of subparts i-x;

(c) the amino acid sequence of subparts (a) or (b) having a methionyl residue at the N-terminus;

(d) the Fc protein, or variant, fragment or derivative thereof, of any of subparts (a) through (c) comprised of a chemical moiety connected to the protein moiety;

(e) a derivative of subpart (d) wherein said chemical moiety is a water soluble polymer moiety;

(f) a derivative of subpart (e) wherein said water soluble polymer moiety is polyethylene glycol; and

(g) a derivative of subpart (e) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

3. The protein according to claim 1, wherein the AGP-1 protein, or variant, fragment or derivative thereof, is selected from the group consisting of:

(a) the amino acid sequence X-281 wherein X is any residue from 95 to 114 inclusive as shown in Figure 2 (SEQ ID NO:34);

(b) the amino acid sequence of subpart (a) having a methionyl residue at the N-terminus.

(c) the AGP-1 protein, or variant, fragment or derivative thereof, of any of subparts (a) and (b) comprised of a chemical moiety connected to the protein moiety;

(d) a derivative of subpart (c) wherein said chemical moiety is a water soluble polymer moiety;

(e) a derivative of subpart (d) wherein said water soluble polymer moiety is polyethylene glycol;

(f) A derivative of subpart (d) wherein said water soluble polymer moiety is a polyamino acid moiety; and

(g) a derivative of subpart (d) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

4. The protein of claim 1 wherein the linker sequence is one or more amino acids selected from the group consisting of: Glycine, Asparagine, Serine, Threonine and Alanine.

5. The protein of claim 1 wherein the linker is selected from the group consisting of:

(a) ala-ala-ala;
(b) ala-ala-ala-ala;
(c) ala-ala-ala-ala-ala;
(d) gly-gly;
(e) gly-gly-gly;
(f) gly-gly-gly-gly-gly;
(g) gly-gly-gly-gly-gly-gly-gly;
(h) gly-pro-gly;
(i) gly-gly-pro-gly-gly;
(j) chemical moiety; and
(k) any combination of subparts (a) through (j).

6. A fusion protein comprising a Fc protein, or variant, fragment or derivative thereof, fused to the N-terminus of an AGP-1 protein, or variant, fragment or derivative thereof.

7. A nucleic acid sequence encoding for a protein having the formula selected from the group consisting of: $R_1 - R_2$ and $R_1 - L - R_2$, wherein R_1 is a Fc protein, or variant or fragment thereof, R_2 is an AGP-1 protein, or variant or fragment thereof, and L is a linker.

8. The nucleic acid sequence according to claim 7 encoding for a protein comprising an Fc protein, variant, fragment or derivative portion selected from the group consisting of:

(a) the Fc amino acid sequences as set forth in Figure 1 (SEQ ID NO: 32);

(b) the amino acid sequence of subpart (a) having a different amino acid substituted or deleted in one or more of the following positions (using the numbering according to Figure 1):

(i) one or more cysteine residues;

(ii) one or more tyrosine residues;

(iii) cysteine at position 5 deleted or substituted with an alanine;

(iv) leucine at position 20 deleted or substituted with glutamine;

(v) glutamic acid at position 103 deleted or substituted with an alanine;

(vi) lysine at position 105 deleted or substituted with an alanine;

(vii) lysine at position 107 deleted or substituted with an alanine;

(viii) deletion or substitution of one or more of the amino acids at positions 1, 2, 3, 4, and 5;

(ix) one or more residues substituted or deleted to ablate the Fc receptor binding site;

(x) one or more residues substituted or deleted to ablate the complement (C1q) binding site; and

(xi) a combination of subparts i-x;

(c) the amino acid sequence of subparts (a) or (b) having a methionyl residue at the N-terminus;

(d) the Fc protein, or variant, fragment or derivative thereof, of any of subparts (a) through (c) comprised of a chemical moiety connected to the protein moiety;

(e) a derivative of subpart (d) wherein said chemical moiety is a water soluble polymer moiety;

(f) a derivative of subpart (e) wherein said water soluble polymer moiety is polyethylene glycol; and

(g) a derivative of subpart (e) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

9. The nucleic acid sequence according to claim 7 encoding for a protein comprising an AGP-1 protein, variant, fragment or derivative portion selected from the group consisting of:

(a) the amino acid sequence X-281 wherein X is any residue from 95 to 114 inclusive as shown in Figure 2 (SEQ ID NO: 34);

(b) the amino acid sequence of subpart (a) having a methionyl residue at the N-terminus.

(c) the AGP-1 protein, or variant, fragment or derivative thereof, of any of subparts (a) and (b) comprised of a chemical moiety connected to the protein moiety;

(d) a derivative of subpart (c) wherein said chemical moiety is a water soluble polymer moiety;

(e) a derivative of subpart (d) wherein said water soluble polymer moiety is polyethylene glycol;

(f) A derivative of subpart (d) wherein said water soluble polymer moiety is a polyamino acid moiety; and

(g) a derivative of subpart (d) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety.

10. The nucleic acid sequence of claim 7 encoding for a protein with a linker sequence of one or more amino acids selected from the group consisting of: Gly, Asn, Ser, Thr and Ala.

11. The nucleic acid sequence of claim 7 encoding for a protein with a linker selected from the group consisting of:

(a) ala, ala, ala;
(b) ala-ala-ala-ala;
(c) ala-ala-ala-ala-ala;
(d) gly-gly;
(e) gly-gly-gly;
(f) gly-gly-gly-gly-gly;
(g) gly-gly-gly-gly-gly-gly-gly;
(h) gly-pro-gly;
(i) gly-gly-pro-gly-gly;
(j) a chemical moiety; and
(k) any combination of subparts (a) through (j).

12. A nucleic acid sequence encoding for a fusion protein having a Fc protein, or variant, fragment or derivative thereof, fused to the N-terminus of an AGP-1 protein, or a variant, fragment or derivative thereof.

13. A vector comprising a nucleic acid sequence according to any of Claims 7 to 12 inclusive.

14. A prokaryotic or eukaryotic host cell containing the vector of claim 13.

15. A process for producing a protein of claims 1 or 6 comprising the steps of culturing, under suitable conditions, the host cell of claim 14, and isolating the protein produced.

16. The process of claim 15 further comprising the step of purifying the protein produced.

17. A pharmaceutical composition comprising an effective amount of a protein according to claims 1 or 6, in a pharmaceutically acceptable diluent, adjuvant or carrier.

18. A method of inducing apoptosis in a tissue comprising administering a therapeutically effective amount of the protein according to Claim 1 or 6.

19. A method of treating of a disorder selected from the group consisting of proliferative disorder, an immune disorder or a viral-induced disorder comprising administering a therapeutically effective amount of the protein according to claims 1 or 6.

FIGURE 1
Amino acid sequence of hinge, CH2 and CH3 regions human IgGγ1

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
```

FIGURE 2
Amino acid and nucleic acid sequence of Fc-huAGP-1 (95-281)

```
GGCTGACTTA CAGCAGTCAG ACTCTGACAG GATC ATG GCT ATG ATG GAG GTC          52
                                      Met Ala Met Met Glu Val
                                       1               5

CAG GGG GGA CCC AGC CTG GGA CAG ACC TGC GTG CTG ATC GTG ATC TTC       100
Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu Ile Val Ile Phe
           10                  15                  20

ACA GTG CTC CTG CAG TCT CTC TGT GTG GCT GTA ACT TAC GTG TAC TTT       148
Thr Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr Tyr Val Tyr Phe
       25                  30                  35

ACC AAC GAG CTG AAG CAG ATG CAG GAC AAG TAC TCC AAA AGT GGC ATT       196
Thr Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser Lys Ser Gly Ile
   40                  45                  50

GCT TGT TTC TTA AAA GAA GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA       244
Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu
55                 60                  65                  70

GAG AGT ATG AAC AGC CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG       292
Glu Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln
                75                  80                  85

CTC GTT AGA AAG ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA       340
Leu Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr
           90                  95                  100

GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT       388
Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly
       105                 110                 115

CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC       436
Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn
   120                 125                 130

ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA       484
Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys
135                140                 145                 150

ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC       532
Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn
                155                 160                 165

TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC       580
Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr
           170                 175                 180

TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA       628
Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu
       185                 190                 195

AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA       676
Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr
   200                 205                 210

AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT       724
Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala Arg Asn Ser Cys
215                220                 225                 230

TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG GGA       772
Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly
                235                 240                 245
```

FIGURE 2 (con't)

```
ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT      820
Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn
            250                 255                 260

GAG CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT      868
Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe
        265                 270                 275

TTA GTT GGC TAA CTGACCTGGA AAGAAAAAGC AATAACCTCA AAGTGACTAT          920
Leu Val Gly  *
    280

TCAGTTTTCA GGATGATACA CTATGAAGAT GTTTCAAAAA ATCTGACCAA AACAAACAAA    980

CAGAAAACAG AAAACAAAAA AACCTCTATG CAATCTGAGT AGAGCAGCCA CAACCAAAAT   1040

TGTATACAAC ACACCATGTA                                              1060
```

FIGURE 3
Fc-huAGP-1(95-281)

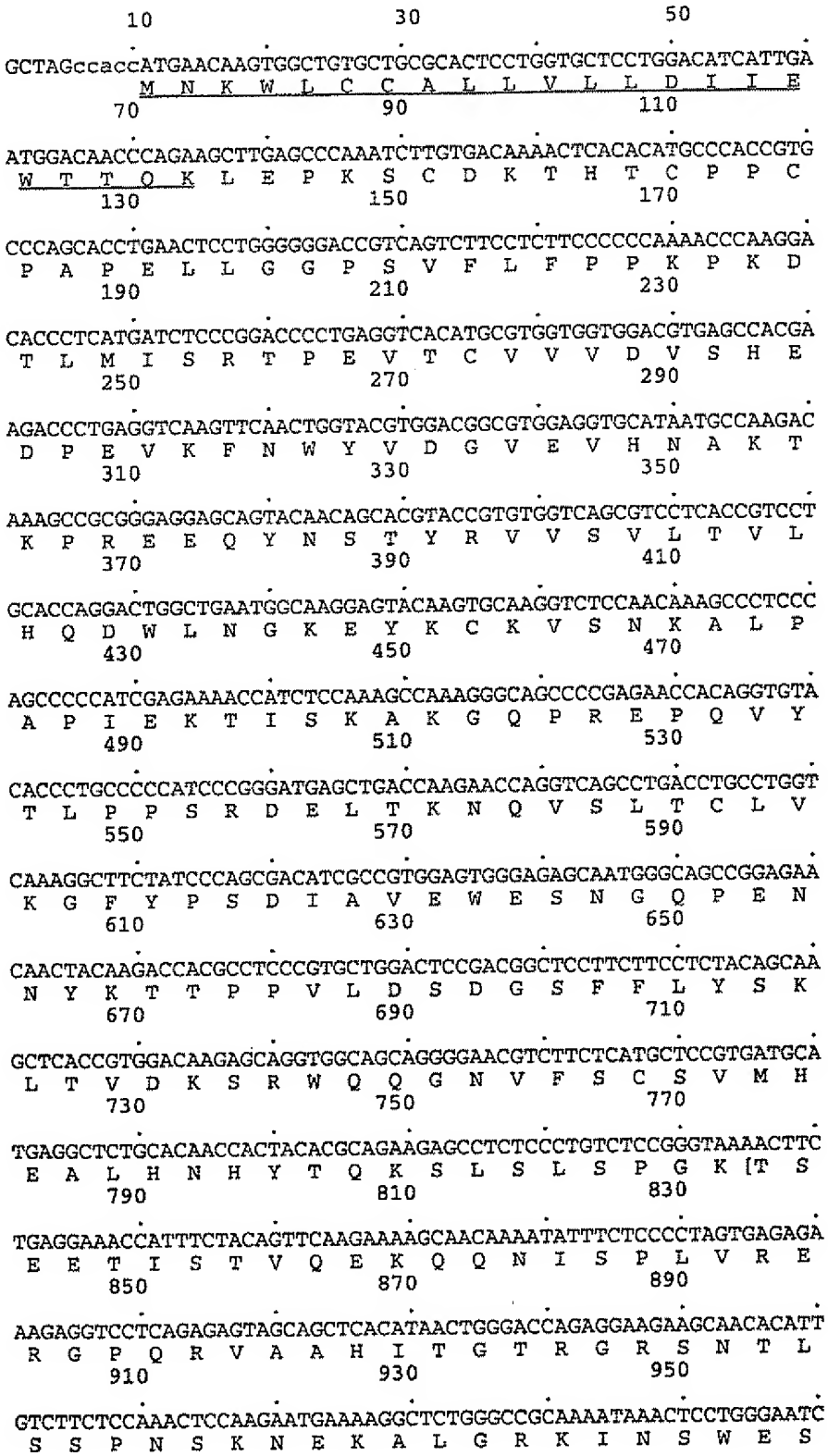


FIGURE 3 (con't)

```
      970                 990                1010
ATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGCGAACTGGTCAT
 S  R  S  G  H  S  F  L  S  N  L  H  L  R  N  G  E  L  V  I
      1030                1050               1070
CCATGAAAAAGGGTTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAAT
 H  E  K  G  F  Y  Y  I  Y  S  Q  T  Y  F  R  F  Q  E  E  I
      1090                1110               1130
AAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATTTACAAATACACAAGTTA
 K  E  N  T  K  N  D  K  Q  M  V  Q  Y  I  Y  K  Y  T  S  Y
      1150                1170               1190
TCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGA
 P  D  P  I  L  L  M  K  S  A  R  N  S  C  W  S  K  D  A  E
      1210                1230               1250
ATATGGACTCTATTCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAAT
 Y  G  L  Y  S  I  Y  Q  G  G  I  F  E  L  K  E  N  D  R  I
      1270                1290               1310
TTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCATGAAGCCAGTTTTTTCGG
 F  V  S  V  T  N  E  H  L  I  D  M  D  H  E  A  S  F  F  G
      1330
GGCCTTTTTAGTTGGCTAActcgag
 A  F  L  V  G] *
```

FIGURE 4
Fc-huAGP-1(114-281)

```
        10                  30                  50
GCTAGccaccATGAACAAGTGGCTGTGCTGCGCACTCCTGGTGCTCCTGGACATCATTGA
          M  N  K  W  L  C  C  A  L  L  V  L  L  D  I  I  E
        70                  90                  110
ATGGACAACCCAGAAGCTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTG
 W  T  T  Q  K  L  E  P  K  S  C  D  K  T  H  T  C  P  P  C
       130                 150                 170
CCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGA
 P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D
       190                 210                 230
CACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGA
 T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E
       250                 270                 290
AGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC
 D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T
       310                 330                 350
AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCT
 K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L
       370                 390                 410
GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
 H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P
       430                 450                 470
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA
 A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y
       490                 510                 530
CACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT
 T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V
       550                 570                 590
CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
 K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N
       610                 630                 650
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAA
 N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K
       670                 690                 710
GCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
 L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H
       730                 750                 770
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGTGAG
 E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K [V  R
       790                 810                 830
AGAAAGAGGTCCTCAGAGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACAC
 E  R  G  P  Q  R  V  A  A  H  I  T  G  T  R  G  R  S  N  T
       850                 870                 890
ATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAAATAAACTCCTGGGA
 L  S  S  P  N  S  K  N  E  K  A  L  G  R  K  I  N  S  W  E
       910                 930                 950
ATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGCGAACTGGT
 S  S  R  S  G  H  S  F  L  S  N  L  H  L  R  N  G  E  L  V
```

FIGURE 4 (con't)

```
      970                 990                1010
       .                   .                  .
CATCCATGAAAAAGGGTTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGA
 I  H  E  K  G  F  Y  Y  I  Y  S  Q  T  Y  F  R  F  Q  E  E
     1030                1050               1070
       .                   .                  .
AATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATTTACAAATACACAAG
 I  K  E  N  T  K  N  D  K  Q  M  V  Q  Y  I  Y  K  Y  T  S
     1090                1110               1130
       .                   .                  .
TTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGC
 Y  P  D  P  I  L  L  M  K  S  A  R  N  S  C  W  S  K  D  A
     1150                1170               1190
       .                   .                  .
AGAATATGGACTCTATTCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAG
 E  Y  G  L  Y  S  I  Y  Q  G  G  I  F  E  L  K  E  N  D  R
     1210                1230               1250
       .                   .                  .
AATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCATGAAGCCAGTTTTTT
 I  F  V  S  V  T  N  E  H  L  I  D  M  D  H  E  A  S  F  F
     1270
       .                   .
CGGGGCCTTTTTAGTTGGCTAActcgag
 G  A  F  L  V  G] *
```

FIGURE 5
Fc-muAGP-1(99-291)

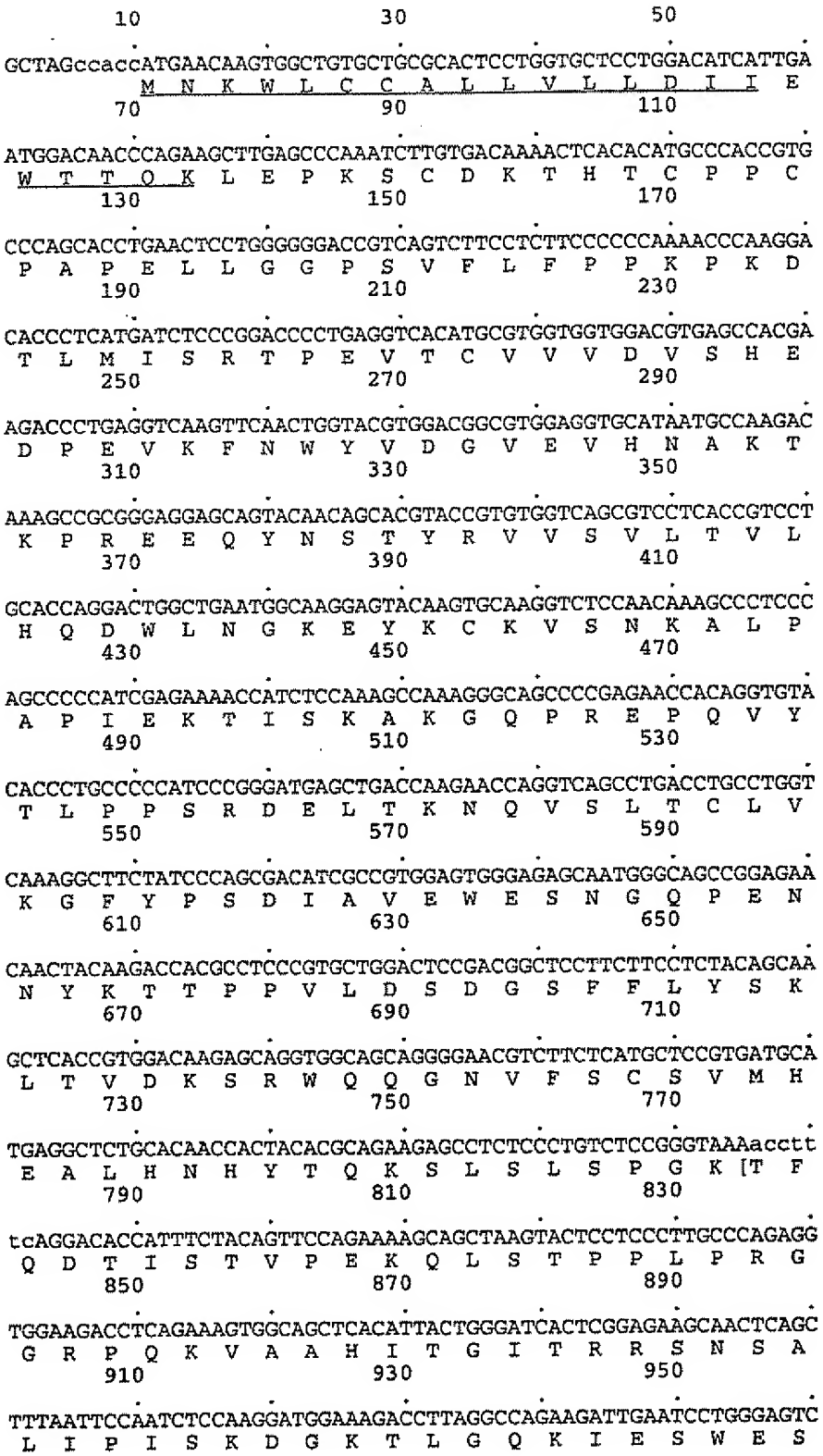


FIGURE 5 (con't)

```
      970                 990                1010
       .                   .                  .
CTCTCGGAAAGGGCATTCATTTCTCAACCACGTGCTCTTTAGGAATGGAGAGCTGGTCAT
 S  R  K  G  H  S  F  L  N  H  V  L  F  R  N  G  E  L  V  I
     1030                1050               1070
       .                   .                  .
CGAGCAGGAGGGCCTGTATTACATCTATTCCCAAACATACTTCCGATTTCAGGAAGCTGA
 E  Q  E  G  L  Y  Y  I  Y  S  Q  T  Y  F  R  F  Q  E  A  E
     1090                1110               1130
       .                   .                  .
AGACGCTTCCAAGATGGTCTCAAAGGACAAGGTGAGAACCAAACAGCTGGTGCAGTACAT
 D  A  S  K  M  V  S  K  D  K  V  R  T  K  Q  L  V  Q  Y  I
     1150                1170               1190
       .                   .                  .
CTACAAGTACACCAGCTATCCGGATCCCATAGTGCTCATGAAGAGCGCCAGAAACAGCTG
 Y  K  Y  T  S  Y  P  D  P  I  V  L  M  K  S  A  R  N  S  C
     1210                1230               1250
       .                   .                  .
TTGGTCCAGAGATGCCGAGTACGGACTGTACTCCATCTATCAGGGAGGATTGTTCGAGCT
 W  S  R  D  A  E  Y  G  L  Y  S  I  Y  Q  G  G  L  F  E  L
     1270                1290               1310
       .                   .                  .
AAAAAAAAATGACAGGATTTTTGTTTCTGTGACAAATGAACATTTGATGGACCTGGATCA
 K  K  N  D  R  I  F  V  S  V  T  N  E  H  L  M  D  L  D  Q
     1330                1350
       .                   .
AGAAGCCAGCTTCTTTGGAGCCTTTTTAATTAACTAActaactcgag
 E  A  S  F  F  G  A  F  L  I  N] *
```

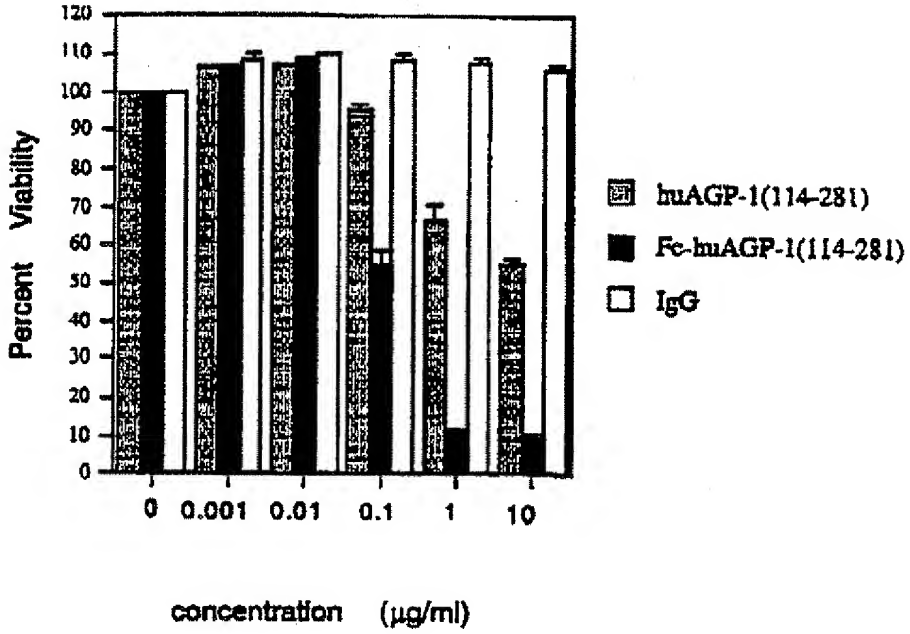
FIGURE 6
Fc-muAGP-1(120-291)

```
        10                  30                  50
        .                   .                   .
GCTAGccaccATGAACAAGTGGCTGTGCTGCGCACTCCTGGTGCTCCTGGACATCATTGA
          M   N   K   W   L   C   C   A   L   L   V   L   L   D   I   I   E
        70                  90                  110
        .                   .                   .
ATGGACAACCCAGAAGCTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTG
 W   T   T   Q   K   L   E   P   K   S   C   D   K   T   H   T   C   P   P   C
        130                 150                 170
        .                   .                   .
CCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGA
 P   A   P   E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   D
        190                 210                 230
        .                   .                   .
CACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGA
 T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H   E
        250                 270                 290
        .                   .                   .
AGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC
 D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N   A   K   T
        310                 330                 350
        .                   .                   .
AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCT
 K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V   L   T   V   L
        370                 390                 410
        .                   .                   .
GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCC
 H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K   A   L   P
        430                 450                 470
        .                   .                   .
AGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA
 A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y
        490                 510                 530
        .                   .                   .
CACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT
 T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C   L   V
        550                 570                 590
        .                   .                   .
CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
 K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N
        610                 630                 650
        .                   .                   .
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAA
 N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K
        670                 690                 710
        .                   .                   .
GCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
 L   T   V   D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H
        730                 750                 770
        .                   .                   .
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGGTGG
 E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K   G   G
        790                 810                 830
        .                   .                   .
AAGACCTCAGAAAGTGGCAGCTCACATTACTGGGATCACTCGGAGAAGCAACTCAGCTTT
 R   P   Q   K   V   A   A   H   I   T   G   I   T   R   R   S   N   S   A   L
        850                 870                 890
        .                   .                   .
AATTCCAATCTCCAAGGATGGAAAGACCTTAGGCCAGAAGATTGAATCCTGGGAGTCCTC
 I   P   I   S   K   D   G   K   T   L   G   Q   K   I   E   S   W   E   S   S
        910                 930                 950
        .                   .                   .
TCGGAAAGGGCATTCATTTCTCAACCACGTGCTCTTTAGGAATGGAGAGCTGGTCATCGA
 R   K   G   H   S   F   L   N   H   V   L   F   R   N   G   E   L   V   I   E
```

FIGURE 6 (con't)

```
                970                 990                1010
                .                   .                  .
GCAGGAGGGCCTGTATTACATCTATTCCCAAACATACTTCCGATTTCAGGAAGCTGAAGA
          Q  E  G  L  Y  Y  I  Y  S  Q  T  Y  F  R  F  Q  E  A  E  D
               1030                1050               1070
               .                   .                  .
     CGCTTCCAAGATGGTCTCAAAGGACAAGGTGAGAACCAAACAGCTGGTGCAGTACATCTA
      A  S  K  M  V  S  K  D  K  V  R  T  K  Q  L  V  Q  Y  I  Y
               1090                1110               1130
               .                   .                  .
     CAAGTACACCAGCTATCCGGATCCCATAGTGCTCATGAAGAGCGCCAGAAACAGCTGTTG
      K  Y  T  S  Y  P  D  P  I  V  L  M  K  S  A  R  N  S  C  W
               1150                1170               1190
               .                   .                  .
     GTCCAGAGATGCCGAGTACGGACTGTACTCCATCTATCAGGGAGGATTGTTCGAGCTAAA
      S  R  D  A  E  Y  G  L  Y  S  I  Y  Q  G  G  L  F  E  L  K
               1210                1230               1250
               .                   .                  .
     AAAAAATGACAGGATTTTTGTTTCTGTGACAAATGAACATTTGATGGACCTGGATCAAGA
      K  N  D  R  I  F  V  S  V  T  N  E  H  L  M  D  L  D  Q  E
               1270                1290
               .                   .
     AGCCAGCTTCTTTGGAGCCTTTTTAATTAACTAActaactcgag
      A  S  F  F  G  A  F  L  I  N] *
```

FIGURE 7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120
110
100
90
80
70
60
50
40
30
20
10
0
0
0.001
0.01
0.1
1
10
concentration (µg/ml)
huAGP-1(114-281)
Fc-huAGP-1(114-281)
IgG

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<110> Amgen Inc.

<120> AGP-1 Fusion Protein Compositions and Methods

<130> A-600

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<141> 1999-04-16

<160> 42

<170> PatentIn Ver. 2.0

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<210> 2
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<212> DNA
<213> Artificial Sequence

<220>
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<400> 2
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<210> 3
<211> 21
<212> PRT
<213> mouse

<400> 3
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1 5 10 15

Glu Trp Thr Thr Gln
20

<210> 4
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
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ggacaaccca ga 72

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ttgttcatgg tg 72

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<212> DNA
<213> Artificial Sequence

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act 63

<210> 11
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<213> Artificial Sequence

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gag 63

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<211> 32

<212> DNA
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<212> DNA
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<210> 24
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic

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<210> 25
<211> 20
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: synthetic

<400> 25
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<212> DNA
<213> Artificial Sequence

<220>
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<210> 27
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic

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<210> 28
<211> 20
<212> DNA
<213> Artificial Sequence

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<400> 28
cttcttcctc tacagcaagc 20

<210> 29
<211> 20
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: synthetic

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<213> Human

<400> 32
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

```
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230


<210> 33
<211> 1060
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                                        Met Ala Met Met Glu Val Gln
                                          1               5

ggg gga ccc agc ctg gga cag acc tgc gtg ctg atc gtg atc ttc aca   103
Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu Ile Val Ile Phe Thr
         10                  15                  20

gtg ctc ctg cag tct ctc tgt gtg gct gta act tac gtg tac ttt acc   151
Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr Tyr Val Tyr Phe Thr
     25                  30                  35

aac gag ctg aag cag atg cag gac aag tac tcc aaa agt ggc att gct   199
Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser Lys Ser Gly Ile Ala
 40                  45                  50                  55

tgt ttc tta aaa gaa gat gac agt tat tgg gac ccc aat gac gaa gag   247
Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu Glu
                 60                  65                  70

agt atg aac agc ccc tgc tgg caa gtc aag tgg caa ctc cgt cag ctc   295
Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln Leu
             75                  80                  85

gtt aga aag atg att ttg aga acc tct gag gaa acc att tct aca gtt   343
Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr Val
         90                  95                  100

caa gaa aag caa caa aat att tct ccc cta gtg aga gaa aga ggt cct   391
```

```
Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly Pro
    105                 110                 115

cag aga gta gca gct cac ata act ggg acc aga gga aga agc aac aca   439
Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn Thr
120                 125                 130                 135

ttg tct tct cca aac tcc aag aat gaa aag gct ctg ggc cgc aaa ata   487
Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys Ile
                140                 145                 150

aac tcc tgg gaa tca tca agg agt ggg cat tca ttc ctg agc aac ttg   535
Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn Leu
            155                 160                 165

cac ttg agg aat ggt gaa ctg gtc atc cat gaa aaa ggg ttt tac tac   583
His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr Tyr
        170                 175                 180

atc tat tcc caa aca tac ttt cga ttt cag gag gaa ata aaa gaa aac   631
Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu Asn
    185                 190                 195

aca aag aac gac aaa caa atg gtc caa tat att tac aaa tac aca agt   679
Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr Ser
200                 205                 210                 215

tat cct gac cct ata ttg ttg atg aaa agt gct aga aat agt tgt tgg   727
Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala Arg Asn Ser Cys Trp
                220                 225                 230

tct aaa gat gca gaa tat gga ctc tat tcc atc tat caa ggg gga ata   775
Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Ile
            235                 240                 245

ttt gag ctt aag gaa aat gac aga att ttt gtt tct gta aca aat gag   823
Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn Glu
        250                 255                 260

cac ttg ata gac atg gac cat gaa gcc agt ttt ttc ggg gcc ttt tta   871
His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe Leu
    265                 270                 275

gtt ggc taactgacct ggaaagaaaa agcaataacc tcaaagtgac tattcagttt    927
Val Gly
280

tcaggatgat acactatgaa gatgtttcaa aaaatctgac caaaacaaac aaacagaaaa 987

cagaaaacaa aaaaacctct atgcaatctg agtagagcag ccacaaccaa aattgtatac 1047

aacacaccat gta                                                    1060
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<210> 34
<211> 281
<212> PRT
<213> Human

<400> 34
Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys

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1               5                  10                  15

Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala
            20                  25                  30

Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys
        35                  40                  45

Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr
    50                  55                  60

Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val
65                  70                  75                  80

Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser
                85                  90                  95

Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro
            100                 105                 110

Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
        115                 120                 125

Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
    130                 135                 140

Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
145                 150                 155                 160

His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
                165                 170                 175

His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
            180                 185                 190

Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
        195                 200                 205

Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys
    210                 215                 220

Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
225                 230                 235                 240

Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
                245                 250                 255

Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
            260                 265                 270

Ser Phe Phe Gly Ala Phe Leu Val Gly
        275                 280
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<210> 35
<211> 1345
<212> DNA
<213> mouse

<220>
<221> CDS

<222> Complement((11)..(1333))

<400> 35

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gctagccacc atg aac aag tgg ctg tgc tgc gca ctc ctg gtg ctc ctg    49
           Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu
             1               5                  10

gac atc att gaa tgg aca acc cag aag ctt gag ccc aaa tct tgt gac    97
Asp Ile Ile Glu Trp Thr Thr Gln Lys Leu Glu Pro Lys Ser Cys Asp
     15                  20                  25

aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga   145
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
 30                  35                  40                  45

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc   193
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                 50                  55                  60

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa   241
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
             65                  70                  75

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat   289
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
         80                  85                  90

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt   337
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
     95                  100                 105

gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag   385
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
110                 115                 120                 125

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag   433
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                130                 135                 140

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac   481
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            145                 150                 155

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg   529
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        160                 165                 170

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg   577
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    175                 180                 185

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg   625
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
190                 195                 200                 205

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac   673
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                210                 215                 220

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat   721
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
```

```
            225                 230                 235

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg   769
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        240                 245                 250

ggt aaa act tct gag gaa acc att tct aca gtt caa gaa aag caa caa   817
Gly Lys Thr Ser Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln
    255                 260                 265

aat att tct ccc cta gtg aga gaa aga ggt cct cag aga gta gca gct   865
Asn Ile Ser Pro Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala
270                 275                 280                 285

cac ata act ggg acc aga gga aga agc aac aca ttg tct tct cca aac   913
His Ile Thr Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn
                290                 295                 300

tcc aag aat gaa aag gct ctg ggc cgc aaa ata aac tcc tgg gaa tca   961
Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser
            305                 310                 315

tca agg agt ggg cat tca ttc ctg agc aac ttg cac ttg agg aat ggc  1009
Ser Arg Ser Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly
        320                 325                 330

gaa ctg gtc atc cat gaa aaa ggg ttt tac tac atc tat tcc caa aca  1057
Glu Leu Val Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr
    335                 340                 345

tac ttt cga ttt cag gag gaa ata aaa gaa aac aca aag aac gac aaa  1105
Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys
350                 355                 360                 365

caa atg gtc caa tat att tac aaa tac aca agt tat cct gac cct ata  1153
Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile
                370                 375                 380

ttg ttg atg aaa agt gct aga aat agt tgt tgg tct aaa gat gca gaa  1201
Leu Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu
            385                 390                 395

tat gga ctc tat tcc atc tat caa ggg gga ata ttt gag ctt aag gaa  1249
Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu
        400                 405                 410

aat gac aga att ttt gtt tct gta aca aat gag cac ttg ata gac atg  1297
Asn Asp Arg Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met
    415                 420                 425

gac cat gaa gcc agt ttt ttc ggg gcc ttt tta gtt ggctaactcg ag    1345
Asp His Glu Ala Ser Phe Phe Gly Ala Phe Leu Val
430                 435                 440
```

<210> 36
<211> 441
<212> PRT
<213> mouse

<400> 36

```
Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu Asp Ile Ile
 1               5                  10                  15

Glu Trp Thr Thr Gln Lys Leu Glu Pro Lys Ser Cys Asp Lys Thr His
            20                  25                  30

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
        35                  40                  45

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
    50                  55                  60

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
65                  70                  75                  80

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                85                  90                  95

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            100                 105                 110

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        115                 120                 125

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    130                 135                 140

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
145                 150                 155                 160

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                165                 170                 175

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
            180                 185                 190

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        195                 200                 205

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
    210                 215                 220

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
225                 230                 235                 240

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Thr
                245                 250                 255

Ser Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser
            260                 265                 270

Pro Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr
        275                 280                 285

Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn
    290                 295                 300

Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser
305                 310                 315                 320

Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val
```

```
                325                 330                 335

Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg
            340                 345                 350

Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val
        355                 360                 365

Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met
    370                 375                 380

Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu
385                 390                 395                 400

Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg
                405                 410                 415

Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu
            420                 425                 430

Ala Ser Phe Phe Gly Ala Phe Leu Val
        435                 440


<210> 37
<211> 1288
<212> DNA
<213> mouse

<220>
<221> CDS
<222> Complement((11)..(1279))

<400> 37
gctagccacc atg aac aag tgg ctg tgc tgc gca ctc ctg gtg ctc ctg    49
           Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu
             1               5                  10

gac atc att gaa tgg aca acc cag aag ctt gag ccc aaa tct tgt gac   97
Asp Ile Ile Glu Trp Thr Thr Gln Lys Leu Glu Pro Lys Ser Cys Asp
     15                  20                  25

aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga   145
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
 30                  35                  40                  45

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc   193
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                 50                  55                  60

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa   241
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
             65                  70                  75

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat   289
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
         80                  85                  90

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt   337
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
     95                  100                 105
```

```
gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag   385
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
110                 115                 120                 125

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag   433
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                130                 135                 140

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac   481
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            145                 150                 155

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg   529
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        160                 165                 170

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg   577
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    175                 180                 185

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg   625
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
190                 195                 200                 205

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac   673
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                210                 215                 220

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat   721
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            225                 230                 235

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg   769
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        240                 245                 250

ggt aaa gtg aga gaa aga ggt cct cag aga gta gca gct cac ata act   817
Gly Lys Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr
    255                 260                 265

ggg acc aga gga aga agc aac aca ttg tct tct cca aac tcc aag aat   865
Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn
270                 275                 280                 285

gaa aag gct ctg ggc cgc aaa ata aac tcc tgg gaa tca tca agg agt   913
Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser
                290                 295                 300

ggg cat tca ttc ctg agc aac ttg cac ttg agg aat ggc gaa ctg gtc   961
Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val
            305                 310                 315

atc cat gaa aaa ggg ttt tac tac atc tat tcc caa aca tac ttt cga   1009
Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg
        320                 325                 330

ttt cag gag gaa ata aaa gaa aac aca aag aac gac aaa caa atg gtc   1057
Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val
    335                 340                 345
```

```
caa tat att tac aaa tac aca agt tat cct gac cct ata ttg ttg atg   1105
Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met
350                 355                 360                 365

aaa agt gct aga aat agt tgt tgg tct aaa gat gca gaa tat gga ctc   1153
Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu
                370                 375                 380

tat tcc atc tat caa ggg gga ata ttt gag ctt aag gaa aat gac aga   1201
Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg
            385                 390                 395

att ttt gtt tct gta aca aat gag cac ttg ata gac atg gac cat gaa   1249
Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu
        400                 405                 410

gcc agt ttt ttc ggg gcc ttt tta gtt ggc taactcgag                1288
Ala Ser Phe Phe Gly Ala Phe Leu Val Gly
    415                 420
```

<210> 38
<211> 423
<212> PRT
<213> mouse

<400> 38

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Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu Asp Ile Ile
  1               5                  10                  15

Glu Trp Thr Thr Gln Lys Leu Glu Pro Lys Ser Cys Asp Lys Thr His
             20                  25                  30

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
         35                  40                  45

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
     50                  55                  60

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 65                  70                  75                  80

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                 85                  90                  95

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            100                 105                 110

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        115                 120                 125

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    130                 135                 140

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
145                 150                 155                 160

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                165                 170                 175

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
```

```
          180                 185                 190

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        195                 200                 205

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
    210                 215                 220

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
225                 230                 235                 240

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Val
                245                 250                 255

Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg
            260                 265                 270

Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala
        275                 280                 285

Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser
    290                 295                 300

Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu
305                 310                 315                 320

Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu
                325                 330                 335

Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile
            340                 345                 350

Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala
        355                 360                 365

Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile
    370                 375                 380

Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val
385                 390                 395                 400

Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe
                405                 410                 415

Phe Gly Ala Phe Leu Val Gly
            420
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<210> 39
<211> 1367
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> Complement((11)..(1354))

<220>
<223> Description of Artificial Sequence: synthetic

<400> 39

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gctagccacc atg aac aag tgg ctg tgc tgc gca ctc ctg gtg ctc ctg    49
           Met Asn Lys Trp Leu Cys Cys Ala Leu Leu Val Leu Leu
             1               5                  10

gac atc att gaa tgg aca acc cag aag ctt gag ccc aaa tct tgt gac    97
Asp Ile Ile Glu Trp Thr Thr Gln Lys Leu Glu Pro Lys Ser Cys Asp
     15                  20                  25

aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga   145
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
 30                  35                  40                  45

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc   193
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                 50                  55                  60

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa   241
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
             65                  70                  75

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat   289
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
         80                  85                  90

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt   337
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
     95                  100                 105

gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag   385
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
110                 115                 120                 125

gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag   433
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                130                 135                 140

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac   481
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            145                 150                 155

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg   529
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        160                 165                 170

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg   577
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    175                 180                 185

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg   625
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
190                 195                 200                 205

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac   673
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                210                 215                 220

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat   721
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            225                 230                 235

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg   769
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```
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        240                 245                 250

ggt aaa acc ttt cag gac acc att tct aca gtt cca gaa aag cag cta   817
Gly Lys Thr Phe Gln Asp Thr Ile Ser Thr Val Pro Glu Lys Gln Leu
    255                 260                 265

agt act cct ccc ttg ccc aga ggt gga aga cct cag aaa gtg gca gct   865
Ser Thr Pro Pro Leu Pro Arg Gly Gly Arg Pro Gln Lys Val Ala Ala
270                 275                 280                 285

cac att act ggg atc act cgg aga agc aac tca gct tta att cca atc   913
His Ile Thr Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro Ile
                290                 295                 300

tcc aag gat gga aag acc tta ggc cag aag att gaa tcc tgg gag tcc   961
Ser Lys Asp Gly Lys Thr Leu Gly Gln Lys Ile Glu Ser Trp Glu Ser
            305                 310                 315

tct cgg aaa ggg cat tca ttt ctc aac cac gtg ctc ttt agg aat gga  1009
Ser Arg Lys Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn Gly
        320                 325                 330

gag ctg gtc atc gag cag gag ggc ctg tat tac atc tat tcc caa aca  1057
Glu Leu Val Ile Glu Gln Glu Gly Leu Tyr Tyr Ile Tyr Ser Gln Thr
    335                 340                 345

tac ttc cga ttt cag gaa gct gaa gac gct tcc aag atg gtc tca aag  1105
Tyr Phe Arg Phe Gln Glu Ala Glu Asp Ala Ser Lys Met Val Ser Lys
350                 355                 360                 365

gac aag gtg aga acc aaa cag ctg gtg cag tac atc tac aag tac acc  1153
Asp Lys Val Arg Thr Lys Gln Leu Val Gln Tyr Ile Tyr Lys Tyr Thr
                370                 375                 380

agc tat ccg gat ccc ata gtg ctc atg aag agc gcc aga aac agc tgt  1201
Ser Tyr Pro Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser Cys
            385                 390                 395

tgg tcc aga gat gcc gag tac gga ctg tac tcc atc tat cag gga gga  1249
Trp Ser Arg Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly
        400                 405                 410

ttg ttc gag cta aaa aaa aat gac agg att ttt gtt tct gtg aca aat  1297
Leu Phe Glu Leu Lys Lys Asn Asp Arg Ile Phe Val Ser Val Thr Asn
    415                 420                 425

gaa cat ttg atg gac ctg gat caa gaa gcc agc ttc ttt gga gcc ttt  1345
Glu His Leu Met Asp Leu Asp Gln Glu Ala Ser Phe Phe Gly Ala Phe
430                 435                 440                 445

tta att aac taactaactc gag                                      1367
Leu Ile Asn
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<210> 40
<211> 448
<212> PRT
<213> Artificial Sequence

<400> 40

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Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
    50                  55                  60

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
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Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
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            100                 105                 110

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        115                 120                 125

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
    130                 135                 140

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
145                 150                 155                 160

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                165                 170                 175

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
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Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
225                 230                 235                 240

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Thr
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Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro Ile Ser Lys Asp
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Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn Gly Glu Leu Val
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Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Arg
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Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Leu Phe Glu
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gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag   385
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acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg   577
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
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gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg   625
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
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ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac   673
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aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat   721
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ggt aaa ggt gga aga cct cag aaa gtg gca gct cac att act ggg atc   817
Gly Lys Gly Gly Arg Pro Gln Lys Val Ala Ala His Ile Thr Gly Ile
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act cgg aga agc aac tca gct tta att cca atc tcc aag gat gga aag   865
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gaa gct gaa gac gct tcc aag atg gtc tca aag gac aag gtg aga acc  1057
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350                 355                 360                 365

ata gtg ctc atg aag agc gcc aga aac agc tgt tgg tcc aga gat gcc   1153
Ile Val Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Arg Asp Ala
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gag tac gga ctg tac tcc atc tat cag gga gga ttg ttc gag cta aaa   1201
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             20                  25                  30

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
         35                  40                  45

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
     50                  55                  60

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 65                  70                  75                  80

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
                 85                  90                  95

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
            100                 105                 110

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
        115                 120                 125

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
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Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
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Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                165                 170                 175

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
            180                 185                 190

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
        195                 200                 205

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
225                 230                 235                 240

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly
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Gly Arg Pro Gln Lys Val Ala Ala His Ile Thr Gly Ile Thr Arg Arg
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Ser Asn Ser Ala Leu Ile Pro Ile Ser Lys Asp Gly Lys Thr Leu Gly
        275                 280                 285

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    290                 295                 300

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Leu Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Ala Glu
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Asp Ala Ser Lys Met Val Ser Lys Asp Lys Val Arg Thr Lys Gln Leu
            340                 345                 350

Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Val Leu
        355                 360                 365

Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Arg Asp Ala Glu Tyr Gly
    370                 375                 380

Leu Tyr Ser Ile Tyr Gln Gly Gly Leu Phe Glu Leu Lys Lys Asn Asp
385                 390                 395                 400

Arg Ile Phe Val Ser Val Thr Asn Glu His Leu Met Asp Leu Asp Gln
                405                 410                 415

Glu Ala Ser Phe Phe Gly Ala Phe Leu Ile
            420                 425
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INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/US 00/08004

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K19/00 C07K14/705 C12N15/86 C12N5/10 A61K38/18
A61P37/00 A61P31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, MEDLINE, CANCERLIT, AIDSLINE, LIFESCIENCES, CHEM ABS Data, WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 01633 A (IMMUNEX CORP) 16 January 1997 (1997-01-16) page 9, line 11 -page 13, line 8 page 21, line 5 -page 24, line 2 examples 8,9,11 claims	1-19
X	WO 97 46686 A (AMGEN INC) 11 December 1997 (1997-12-11) cited in the application page 3, line 13-24 claims 14-19 page 10, line 2-31	1,3,6,7, 9,12-17, 19

-/--

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2000	27/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/08004

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SHERIDAN J P ET AL: "Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 277, 8 August 1997 (1997-08-08), pages 818-821, XP002065023 ISSN: 0036-8075 the whole document	1-19
A	KIM J -K ET AL: "IDENTIFYING AMINO ACID RESIDUES THAT INFLUENCE PLASMA CLEARANCE OF MURINE IGG1 FRAGMENTS BY SITE-DIRECTED MUTAGENESIS" EUROPEAN JOURNAL OF IMMUNOLOGY,DE,WEINHEIM, vol. 24, no. 3, 1 January 1994 (1994-01-01), pages 542-548, XP000590871 ISSN: 0014-2980 the whole document	2,8
A	DANILENKO D M (REPRINT) ET AL: "AGP - 1, a novel member of the tumor necrosis factor family, induces hepatic necrosis and inflammation in transgenic mice." FASEB JOURNAL, (28 FEB 1997) VOL. 11, NO. 3, PP. 2951-2951, XP002141678 the whole document	1-19

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/08004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9701633 A	16-01-1997	AU 5596599 A	13-01-2000
		AU 708239 B	29-07-1999
		AU 6340796 A	30-01-1997
		CA 2225378 A	16-01-1997
		EP 0835305 A	15-04-1998
		JP 11508445 T	27-07-1999
		NO 976045 A	02-03-1998
		NZ 311982 A	30-08-1999
		US 5763223 A	09-06-1998
WO 9746686 A	11-12-1997	AU 3381097 A	05-01-1998
		CA 2256464 A	11-12-1997
		EP 0918860 A	02-06-1999